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# Experimental Studies on the Production and Treatment of the Carotid Thrombosis in Dogs

—Especially on the Application of Fibrinolytic Treatment—

by

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## INTRODUCTION

Surgical reconstructive operations, such as thromboendarterectomy or graft operation, have been widely recognized as the only mode of treatment which assures the immediate restoration of thrombotic or embolic occlusion of the vessels. However, such a reconstructive operation is usually impossible in the intracranial vessels. Moreover, among the cases of operable extracranial lesions, such as those of the internal carotid and vertebral arteries, circulation is successfully restored in 90 per cent of cases with the partial occlusion but in only 30 per cent with complete occlusion of the vessels<sup>7)</sup>. Therefore, if the intravascular clot is dissolved by the fibrinolytic enzymes and the circulation is restored without accompanying any irreversible damages in the brain, fibrinolytic enzymes might be used more widely in combination with the surgical treatment.

The clinical use of the fibrinolytic enzymes by intravascular injection has recently been investigated and the feasibility of this treatment has been proved in cases with thromboembolic diseases, such as peripheral, pulmonary and coronary vascular occlusions, etc.<sup>4) 5) 6) 10) 14) 18) 25)</sup>. In cerebral vascular occlusive diseases, however, the enzymatic treatment has not yet generally been accepted as the treatment of choice for several reasons, such as 1) the difficulty in clinical differentiation between cerebral vascular occlusions and cerebral hemorrhage, and 2) the danger of hemorrhage into the infarcted area that might be caused by the degeneration of the vessel wall distal to the occlusion and then subsequent dissolution of the clot even in cases with cerebrovascular occlusions, etc.

In 1962 J. HANDA<sup>12)</sup> in our laboratory has demonstrated in dogs that consistent focal cerebral softenings can be produced in high percentage by the production of mural thrombus in the common carotid artery or the introduction of fresh homologous clots into the internal carotid artery after clipping of the posterior communicating and anterior cerebral arteries, and that such cerebral softenings may be prevented by the intravenous injection of the fibrinolytic enzymes without inducing or intensifying further intracerebral hemorrhage.

However, there still remain several problems which should be elucidated before the fibrinolytic enzymes are used in clinical cases. These consist of 1) dosage and mode of applications, 2) timing of administration, 3) complications and 4) combined use with surgery and/or anticoagulant drugs, etc.

The present study has been attempted to elucidate these problems in cases with carotid thrombosis produced in dogs.

## PART I. PRODUCTION OF EXPERIMENTAL CAROTID THROMBOSIS IN DOGS

It is well known that an experimental thrombosis in either artery or vein is produced by the combination of the following 3 factors; 1) injury to the intima of the vascular wall, 2) stasis or turbulence of the blood flow and 3) hypercoagulability of the blood in the general or local circulation. Moreover, it is also true that arterial thrombosis is much difficult to produce than venous thrombosis especially due to the difference of the rate of blood flow.

Taking these facts into consideration, a pair of constricting ligatures were at first placed around the carotid artery to make stagnation or turbulence of blood flow and then the following 4 methods were attempted; 1) chemical intimestomy, 2) mechanical intimestomy, 3) thrombin injection and 4) electric production of thrombus.

### METHODS

Adult mongrel dogs, all weighing 7 to 12kg., were used. Anesthesia was maintained by intravenous Nembutal. Under aseptic precautions, the common carotid artery with its superior thyroid branch was exposed, and carefully dissected free from the surrounding tissues. Two silk ligatures were tied around the carotid artery together with a No. 18 needle placed parallel and attached to the common carotid artery, at a distance of approximately 3.5cm. Then the needle was gently slipped out of the loop, and constricting ligatures with constant diameter were left around the common carotid artery. The superior thyroid artery was always included between these ligatures. A segment of the common carotid artery was isolated between 2 bulldog clamps placed just distal and proximal to the constricting ligatures.

Method I. (Chemical Intimestomy) : Through the previously dissected superior thyroid branch, a No. 24 needle was introduced into the segment. All blood was aspirated from this portion of the vessel, and then 0.3 to 0.5ml. of 5% sodium morrhuate solution was filled through the needle. After 2 minutes, the vessel was flushed with saline solution repeatedly. Both the needle and clamps were removed and the branch used as the needle inlet was ligated.

Method II. (Mechanical Intimestomy) : After the superior thyroid branch was ligated, a longitudinal arteriotomy of about 5mm. in length was made at the proximal end of the isolated segment. The intima was thoroughly stripped out over a 2cm. segment by a hypodermic needle with a bent point. The arteriotomy was closed with No. 3-0 arterial silk. After removal of the bulldog clamps, bleeding from the arterial suture was controlled easily with gentle finger pressure.

Method III. (Thrombin Injection) : By using a No. 24 needle introduced through the dissected superior thyroid branch, the blood in the isolated segment was aspirated into a syringe containing 0.1ml. of bovine thrombin solution (500 units/ml. saline) and rapidly reinjected into the same segment. After waiting for 10 minutes, the bulldog

clamps were removed and the branch was ligated.

Method IV. (Electric production of thrombi) : A blood flow was maintained throughout the procedure, and thrombosis was produced by the passage of the electric current between two, internal and external, electrodes. The internal electrode was made of a fine platinum wire about 5cm. in length attached to a copper lead and insulated by polyethylene tube, and the external electrode made of platinum plate about 40mm. square in area attached to a copper wire (Fig. 1). The voltage source consisted of five 1.5 volt dry cells attached in series to a milliammeter. The internal electrode was inserted proximally into the lumen of the vessel through the previously dissected branch, and the external electrode was closely approximated to the adventitia of the vessel (Fig. 2). The segment through which the current would be passed was carefully insulated from the surrounding tissues by a rubber dam in an effort to minimize electrical leak, and covered by wet cotton to prevent dehydration of the vessel wall. Three milliampere current was passed through the vessel wall from the internal to the external electrode for 60 minutes. After passage of the current both the electrodes and the constricting ligatures were removed and the branch used as the inner electrode inlet was ligated.

All preparations in the above-mentioned 4 methods were examined precisely by palpation or opening the vessel lumen to know the extent and type of thrombus formed at varying times after the procedures.

## RESULTS

Results of these experiments are shown in Tables 1 to 4.

Method I. (Chemical Intimectomy) : Eight vessels were examined on the 3rd day. Of these, 3 were totally occluded and 5 were found to be open. Another 8 vessels were examined on the 5th day and showed the same results as examined on the 3rd day. Thus, the total thrombosis rate was only 37.5 per cent (Table 1).

Method II. (Mechanical Intimectomy) : Eight vessels were examined on the 3rd day.

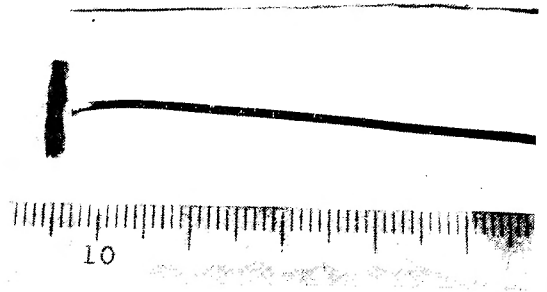


Fig. 1. Two electrodes (upper ; an internal and lower ; an external electrode) used for the electric production of thrombus.

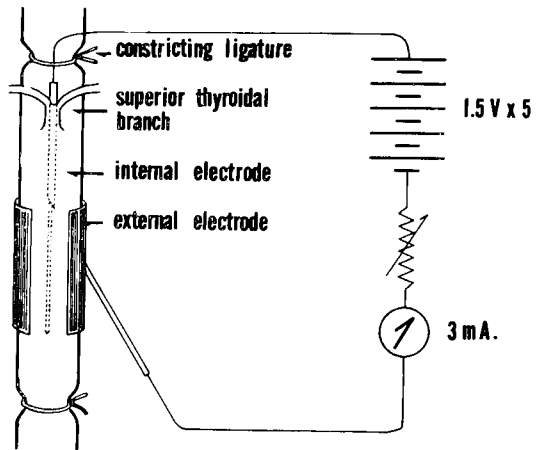


Fig. 2. Schematic illustration of the method of electric production of thrombus.

Of these, 4 showed a complete occlusion, 1 had a mural thrombus and 3 were patent. Another 8 vessels were examined on the 5th day. Of these, 3 were completely occluded and 5 showed no evidence of clot formation. Thus, the incidence of thrombosis including mural and occluding thrombus was only 50.0 per cent in this group (Table 2).

Method III. (Thrombin Injection) : Eight vessels were examined on the 3rd day. Of these, 5 showed a complete occlusion and 3 were found to be open. Of another 8 vessels examined on the 5th day, 5 had an occluding thrombus, 1 mural thrombus and 2 were patent. Thus, the thrombosis rate was 68.8 per cent in this group (Table 3).

Method IV. (Electric Production of Thrombus) : Ninety-nine vessels were included in this study. Ninety of the 99 vessels were examined by palpation immediately after the passage of electric current. Of these, 35 showed an occluding thrombus, 54 mural thrombus and 1 found to be open. The thrombosis rate was 98.9 per cent when examined immediately after the procedures.

Thirty of the 99 vessels were removed on the 2nd day. Of these, 24 had an occluding, 3 had a mural thrombus and the remaining 3 were patent. Of the 46 vessels removed on the 3rd day, 41 were completely occluded, 1 partially occluded and 4 had no thrombus. The remaining 23 of the 99 vessels were removed at varying times after the 4th day. Of them, 20 were completely occluded, 1 partially occluded and 2 were patent. The total thrombosis rate was 90.9 per cent when examined on the 2nd to 32nd day (Table 4).

**Table 1** Incidence of thrombosis following chemical intinectomy (Method I)

Time of Examination	No. of Vessels	Mural Thrombus	Occluding Thrombus	Thrombosis Rate
3rd day	8	0	3	37.5%
5th day	8	0	3	37.5%
Totals	16	0	6	37.5%

**Table 2** Incidence of thrombosis following mechanical intinectomy (Method II)

Time of Examination	No. of Vessels	Mural Thrombus	Occluding Thrombus	Thrombosis Rate
3rd day	8	1	4	62.5%
5th day	8	0	3	37.5%
Totals	16	1	7	50.0%

**Table 3** Incidence of thrombosis following thrombin injection (Method III)

Time of Examination	No. of Vessels	Mural Thrombus	Occluding Thrombus	Thrombosis Rate
3rd day	8	0	5	62.5%
5th day	8	1	5	75.0%
Totals	16	1	10	68.8%

**Table 4** Incidence of thrombosis following electric production of thrombus (Method IV)

Time of Examination	No. of Vessels	Patent Vessel	Mural Thrombus	Occluding Thrombus	Thrombosis Rate
Immediately after Formation	90	1	54	35	98.9%
2nd day	30	3	3	24	90.0%
3rd day	46	4	1	41	91.3%
4-5th day	11	2	0	9	81.8%
6-8th day	7	0	1	6	100 %
24-32nd day	5	0	0	5	100 %
Totals	99	9	5	85	90.9%

COMMENTS

I. Comparison between 4 methods for producing carotid thrombosis.

The most important prerequisite for studying the treatment of carotid thrombosis in animals is to establish a method by which a constant and standardized thrombosis is produced in high percentage. Many technics<sup>9)</sup> have been reported for producing the experimental thrombosis in arteries; however, none of these technics have met such a prerequisite.

As mentioned, the percentage of thrombosis was 37.5 per cent in method I (chemical intinectomy), 50.0 per cent in method II (mechanical intinectomy), 68.8 per cent in method III (thrombin injection) and 90.9 per cent in method IV (electric production of thrombus), respectively. Thus, the percentages of thrombosis are relatively low in the first 3 methods (I III); moreover, the thrombus produced by these methods is likely to be dissolved spontaneously with the elapse of days, usually within 2 weeks. Thus, these 3 methods are considered to be unsatisfactory for the present study.

On the contrary, the percentage occurrence of thrombosis is considerably high in method IV and the thrombus produced by this method has no tendency to be dissolved spontaneously. Moreover, it is composed of the animal's own blood and does not contain contaminants, such as bovine thrombin or bovine fibrinogen. In the present study, therefore, only this method is used in the following experiments.

II. Electric production of thrombus.

The vessel wall has an electrical potential difference with the vessel intima normally negative in respect to the adventitia. SAWYER and PATE<sup>19)20)</sup> found in studies of injury current in the aorta of dogs that a thrombus frequently occurred when the normal potential of the vessel wall was altered by the intimal damage and that a small current flow across the vessel wall in direction to reverse the normal polarity was followed by thrombosis.

Since the report of SAWYER and PATE, several authors<sup>21)</sup> have attempted to produce thrombosis by modifying this technic variously. However, as all of them used only an external electrode, much more electric current had to be applied to produce an arterial thrombus. In the present study, therefore, two electrodes, an internal and external, were

used in combination with a pair of constricting ligatures. After examining the amperage and the duration of passing a 3 milliamperage current for 1 hour not only produced the most reproducible thrombus, but also gave little damage to the vessel wall.

#### *Characteristics of the electric thrombus.*

As shown in Fig. 3, almost two thirds of the electric thrombi were mural immediately after thrombus formation, and then rapidly grew up to occlude the vessel lumen completely. Thus, a 89.1 per cent of the vessels showed complete occlusion when examined on the 3rd day, and moreover the rate of occluding thrombosis maintained almost constancy thereafter. Figure 4 shows a macroscopic appearance of the occluding thrombus of 3 days old. Microscopic sections of the vessels with mural and complete occlusions are shown in Figures 5 and 6.

Microscopic examination of the thrombus showed that aggregates of fibrin and platelets in a meshwork pattern were mainly located in the center of the vessel lumen where the inner electrode had been placed, and surrounded by the remaining blood

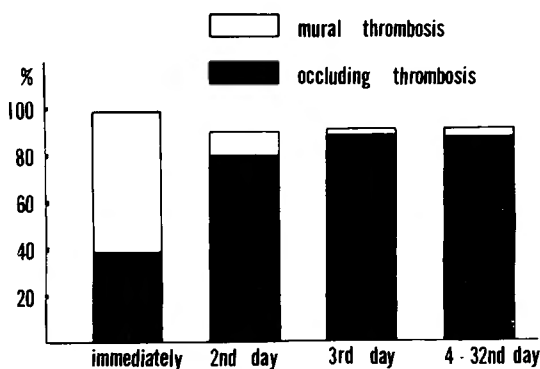


Fig. 3. Histogram of the percentage occurrence of mural and occluding thrombosis, clearly showing the rapid growth from mural to occluding thrombus.

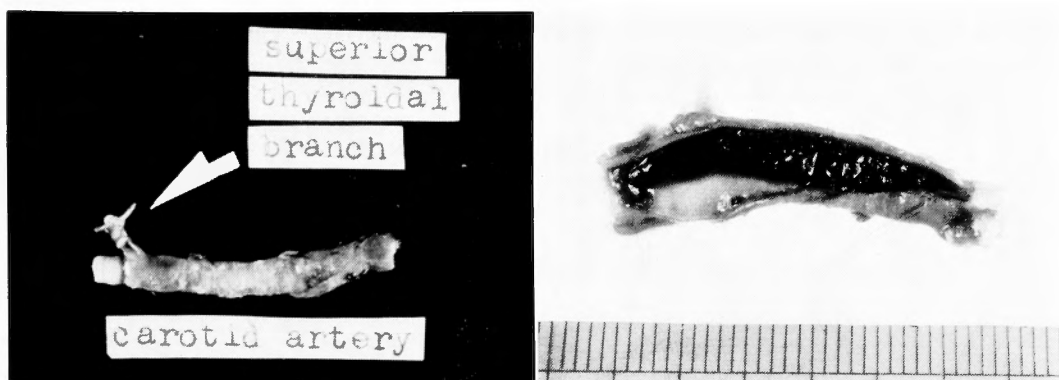


Fig. 4. Macroscopic appearance of the electric thrombus of 3 days old. Left; an external appearance. Right; inside of vessel lumen.

elements which were incorporated in successive laminae (Fig. 7). With the elapse of days, red thrombi may gradually extend distally as well as proximally in such an original pattern of thrombi, forming complete obstructing thrombi.

A small yellowish plaque was sometimes observed in a corresponding area of the intima where the external electrode had been placed. However, as shown in Fig. 8, such an intimal change appears to be considerably small as compared with the changes induced by other methods.

At any rate, such an electric thrombus is a so-called platelet thrombus and has no

tendency to be dissolved spontaneously. These facts seem to indicate that this type of thrombus is difficult to be dissolved by an ordinal enhanced fibrinolytic activity.

### SUMMARY

1) Four different methods, such as chemical intimestomy, mechanical intimestomy, thrombin injection and electric production of thrombus, have been attempted to produce carotid thrombosis in dogs.

2) The percentage of thrombosis was 37.5 per cent in chemical intimestomy, 50.0 per cent in mechanical intimestomy, 68.8 per cent in thrombin injection and 90.9 per cent in electric production of thrombus, respectively. Thus, the method of electric production of thrombus was found to be the most suitable method for studying the present study.

3) Almost two-thirds of the electric thrombi were mural immediately after the procedure and then gradually grew up or extended to occlude vessel lumen completely usually within 48 hours.

4) This electrically produced thrombus is composed of the dog's own blood and does not contain contaminants such as bovine thrombin or bovine fibrinogen. Moreover, this thrombus is a so-called platelet thrombus and has no tendency to be dissolved spontaneously.

## PART II. FIBRINOLYTIC AND SURGICAL THERAPY FOR EXPERIMENTAL CAROTID THROMBOSIS IN DOGS

In part I an electric production of thrombus has been found to be the most suitable method for producing complete occluding thrombosis in the carotid artery in dogs. Using such a thrombus, the following studies were done to find out the most effective method for restoring circulation of the carotid artery.

### PRELIMINARY EXPERIMENTS

There are two controversial opinions on the mechanism by which a thrombus is lysed

### SCHEMES OF CLOT LYSIS

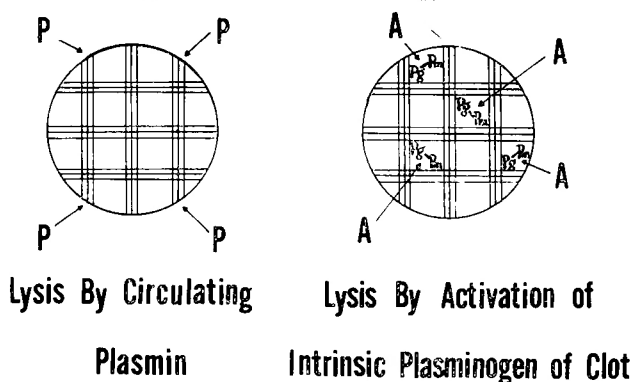


Fig. 9. Schemes of clot lysis (SHERRY). P : Circulating plasmin. A : Circulating activator. Pg-Pm : Activation of intrinsic plasminogen to plasmin.

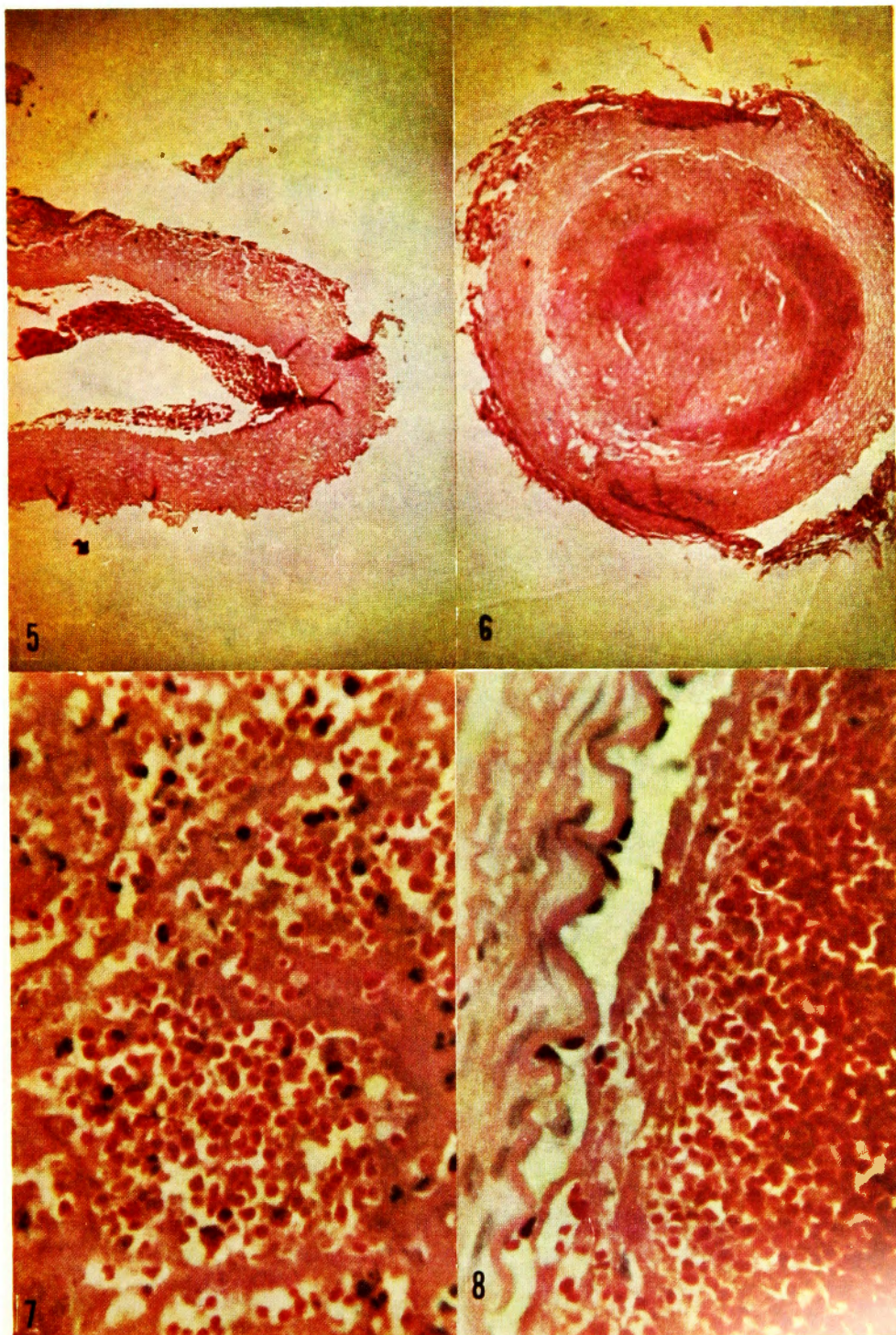


**Fig. 5.** Microscopic section taken 24 hours after electric production of thrombus. Note the mural thrombus adherent to the intima.

**Fig. 6.** Microscopic section taken 1 week after electric production of thrombus. Note the complete occlusion of the vessel lumen.

**Fig. 7.** Microscopic section of the electric thrombus. Note the aggregates of fibrin and platelets in a meshwork pattern surrounding the remaining blood elements.

**Fig. 8.** Microscopic section of the electric thrombus and vessel wall. Note changes of the intima are minimal.



(Fig. 9). One of which, asserted by AMBRUS *et al.*<sup>1)16)</sup>, is that clot lysis results from the direct activation of circulating plasmin or fibrinolysin on the fibrin meshwork of a thrombus, and thus the concentration of circulating fibrinolysin determines the rate of fibrinolysis. Another assumption, asserted by SHERRY *et al.*<sup>23)24)</sup>, is that clot lysis results from the diffusion of activator into the thrombus with resultant activation of the intrinsic profibrinolysin, followed by lysis of the clot, and thus the level of activator in the circulation rather than the level of circulating fibrinolysin, becomes the decisive factor in controlling the rate of lysis of a thrombus. Which assumption is true, has not yet been elucidated. However, it is generally accepted that artificial thrombolysis can be induced in two ways; 1) by injection of streptokinase in the blood stream, which lyses a clot by the internal thrombolysis, and 2) by injection of plasmin, which lyses a clot by the external thrombolysis.

Taking these facts into consideration, intravenous injection of streptokinase added with a small amount of human plasma<sup>22)</sup> was attempted in the present study.

It is also known that there is a considerable variation in the content of the antibody to streptokinase in individuals. In this preliminary experiments, therefore, predictive dose tests were made in normal 6 dogs to determine the amount of streptokinase which had to satisfy all the circulating antibody and then maintain a free level of circulating streptokinase in order to induce an active fibrinolytic state.

**PREDICTIVE DOSE TESTS:** Serial dilutions of streptokinase, such as 500, 250, 200, 150, 100 units per ml., were prepared. Two ml. of each dilution was mixed with 1ml. of dilution of desiccated human plasma containing 0.05ml. of normal human plasma constituent and was incubated for 30 minutes at room temperature. Then, 1ml. aliquots of freshly drawn canine blood were collected in small glass test tubes and 0.3ml. of each dilution of streptokinase-human plasma mixtures was added. In other words, 1ml. of the canine blood and 0.005 ml. of human plasma were mixed with 100, 50, 40, 30 or 20 units streptokinase, respectively. As shown in Table 5, 30 to 40 units of streptokinase was required to produce clot lysis of 1 ml. blood in approximately 20 minutes in 5 out of 6 dogs. This dose was called as 20 minutes test end point<sup>9)</sup> With 100 units of streptokinase, blood remained uncoagulable in 4 dogs during 2 hours observation.

**METHODS OF STREPTOKINASE ADMINISTRATIONS:** Two different dosages of streptokinase injections were planned.

**Table 5** Predictive dose test in 6 normal animals, showing 30 to 40 units of SK. is required to produce clot lysis of 1ml. blood in about 20 minutes. With 100 units of SK. blood remains uncoagulable in most of the animals during 2 hours.

SK. Unit	Dog No.					
	Lysis Time					
	No.101	No.102	No.103	No.104	No.105	No.106
100	no clot	18 min.	15 min.	no clot	no clot	no clot
50	13 min.	21 min.	15 min.	10 min.	no clot	no clot
40	13 min.	27 min.	19 min.	16 min.	15 min.	19 min.
30	21 min.	33 min.	27 min.	18 min.	23 min.	19 min.
20	55 min.	2 hrs.	97 min.	2 hrs.	2 hrs.	2 hrs.

1) *Small Dosage of SK.*: The initial streptokinase dose required for each dog was calculated by multiplying 30 or 40 units of SK. with 0.005 ml. of human plasma by the dog's calculated total blood volume. This initial dose was dissolved in 80 ml. of physiological saline solution and injected into the femoral vein over a 30 minutes period. Thereafter, a sustaining infusion was given at the rate of a half amount of initial dose per hour over a 3 hours period.

2) *Larger Dosage of SK.*: In this method of injection, the initial SK. dose was calculated by multiplying 100 units of SK. with 0.005ml. of human plasma by the calculated total blood volume. Both the initial and sustaining infusions were given in the same way as those of small dosage of SK.

**BIOCHEMICAL STUDIES**: Blood samples were taken before, and at various time intervals after streptokinase injections and the following biochemical studies were made; thrombelastographic studies on whole blood, fibrinogen levels, Lee-White clotting time and subsequent lysis time, euglobulin lysis time and fibrin plate method.

The standard fibrin plates were made according to ASTRUP's technic<sup>2)13)15)</sup>. The plates were either used as such or heated at 85 degree (C.) for 30 minutes. Plasma was separated by centrifugation of citrated blood sample at 2,500 rpm. for 10 minutes. Euglobulin fraction at pH 5.2 was obtained by adding one per cent acetic acid to the plasma. The relative areas of lysis obtained from the euglobulin fraction on the standard plates were evaluated as activator activity, and those on the heated plates as plasmin activity. To evaluate plasminogen activity, 0.5ml. of the euglobulin fraction was incubated for 30 minutes at room temperature with 0.1 ml. of human activator in order to convert plasminogen to plasmin. The human activator was prepared by mixing equal volumes of streptokinase (1000 units per ml.) and human euglobulin. The lytic areas of these preparations on the heated plates were evaluated as whole plasmin activities. The remainder of the plasmin from whole plasmin activity was regarded as plasminogen activity. To test inhibitor activity, 0.03ml. drops of plasma was put over the equal volume of Fibrinolysin (Merck) containing 1000 MSD. units per ml. on the heated plate. As the results, inhibitor in the plasma controlled the lysis of the standard Fibrinolysin. Then, the inhibitor activity was calculated as follows:

Inhibitor =  $A - B / A$ , A : Lytic areas of Fibrinolysin.

B : Lytic areas of Fibrinolysin inhibited by plasma.

## METHODS

Mongrel dogs ranging in weight from 7 to 13 kg. were anesthetized with Nembutal. In the common carotid artery of each dog the electric clot was produced by a previously described method. At varying times after thrombus formation, either within 1 hour, on the 2nd or 3rd day, the animals were reanesthetized and the following 2 series of treatments, fibrinolytic and surgical, were performed.

### SERIES I.: FIBRINOLYTIC TREATMENTS

(Group 1) : Intravenous injection of small dosage of SK.

The effect of treatment with small dosage of SK. was studied on both electric and thrombin clots. Within 1 hour after clot formation, small dosage of SK. was administered

intravenously by a method described. The total amounts of SK. used in each dog ranged from 45,000 to 68,000 units.

(Group 2) : Repeated treatment with small dosage of SK. for 3 days.

A treatment similar to that described in group 1 was repeated once daily for 3 days. This series of treatment was started either within 1 hour or on the 2nd day following thrombus formation. The total amounts of SK. given in each animal ranged from 210,000 to 330,000 units.

(Group 3) : Intravenous injection of larger dosage of SK.

Intravenous injection of larger dosage of SK. was given by a method described within 1 hour, on the 2nd or 3rd day after clot formation. The total amounts of SK. used in each animal ranged from 135,000 to 230,000 units.

(Group 4) : Combined use of repeated small dosage of SK. and heparin.

Intramuscular injection of heparin was combined with repeated treatment with small dosage of SK. The initial heparin dose, 2 mg. per kg., was given 30 minutes prior to SK.-therapy, and then the same dose was also given every 8 hours until the time SK.-therapy for 3 days was completed.

(Group 5) : Combined use of larger dosage of SK. and heparin.

Heparin was given intramuscularly 30 minutes prior to the injection of larger dosage of SK. and then the same dose of heparin was also given every 8 hours over a period of 48 hours.

Animals in all groups were reanesthetized 7 days later and the vessels were removed to examine the extent and type of a thrombus remained.

The results of treatments were evaluated as the following 5 grades ; 1) Grade 0. : No clot remained in the vessel. 2) Grade I. : Only a membranous mural thrombus adherent to the intima was found. 3) Grade II. : There was a mural thrombus occluding less than half of the vessel lumen. 4) Grade III. : A mural thrombus occluding more than half of the vessel lumen was still found. 5) Grade IV. : A complete obstructing thrombus was still found in the vessel.

In each group, a percentage of circulation restored was calculated by regarding grades 0. to II. as restored circulation.

## SERIES II. SURGICAL TREATMENTS

This series was studied to compare the effectiveness of thrombectomy with that of fibrinolytic treatment, and also to find out the most effective method for preventing clot reformation after thrombectomy.

(Group 6) : Thrombectomy.

Thrombectomy was performed by a following method: A thrombosed segment of the artery was isolated between 2 bulldog clamps. A longitudinal arteriotomy of approximately 8 mm. in length was made at the distal part of the isolated segment where the adventitia had been previously stripped out. Then the clot in this segment was thoroughly removed and the vessel lumen was flushed with saline solution containing 20 units of heparin per ml. The arteriotomy was closed with a continuous suture of 3-0 arterial silk. Each suture was made as close as possible to ensure a complete closure. After removal of the clamps, bleeding from the arteriotomy was controlled with gentle

finger pressure.

The above-mentioned procedure was performed within 1 hour, on the 2nd or 3rd day after clot formation.

(Group 7) : Coating with EDH-Adhesive after thrombectomy.

Some animals in group 5 died from loss of blood due to rupture of the vessel wall several days after thrombectomy. To prevent such an arterial rupture, the thrombectomized arteries were coated with EDH-Adhesive, which was found to be the most effective agent for coating and reinforcing intracranial aneurysms<sup>(11)(17)</sup>. This procedure was done on clots of 1 hour, the 2nd or 3rd day of formation.

This group was studied as a control for the following 3 groups.

(Group 8) : Anticoagulation with heparin after thrombectomy.

After coating the thrombectomized artery with EDH-Adhesive, heparin was given intramuscularly at a dose of 2mg. per kg., and then the same dose was also given every 8 hours during the first 48 hours.

(Group 9) : Intramuscular administration of streptokinase after thrombectomy.

Intramuscular administration of streptokinase was given into animals in which coating with EDH-Adhesive had been done after thrombectomy. Dosage of streptokinase for a single injection was calculated by multiplying 30 units of SK. with 0.005 ml. of human plasma by the animal's calculated blood volume. This dose was dissolved in 5 ml. of physiological saline solution and injected intramuscularly 30 minutes prior to thrombectomy, and then the same dose was given every 8 hours for 48 hours.

(Group 10) : Combined use of streptokinase and heparin after thrombectomy.

Both streptokinase and heparin were given intramuscularly every 8 hours in the same manner as described after thrombectomy.

Animals employed in this series were reanesthetized 7 days after the initiation of therapy, the vessels were removed and examined.

## RESULTS

### SERIES I. : FIBRINOLYTIC TREATMENT.

(Group 1) : Intravenous injection of small dosage of SK.

The results of this group are shown in Table 6.

Six vessels with electric clots were treated within one hour of formation. Of these, circulation was restored in only 1 and the remaining 5 were found to be completely occluded. In contrast, 3 out of 4 thrombin clots were completely dissolved when treated within 1 hour of formation. The dissolution rate of electric clots was only 16.7 per cent, while that of thrombin clots was 75.0 per cent.

**Table 6** Results of treatment with small dosage of SK. (Group 1)

Type of Thrombus	No. of Cases	Grade of Occlusion					Percentage of Circulation restored
		IV.	III.	II.	I.	0.	
Electric	6	5		1			16.7%
Thrombin	4	1				3	75.0%



Figure 10 shows the changes in fibrinolytic activity in animals treated with this dose of SK. This seems to indicate that as streptokinase concentration in plasma increases, plasma plasminogen is rapidly activated to plasmin and the plasminogen concentration falls to almost zero within the first 30 minutes of infusion. Then both the activator and plasmin concentration reaches a relatively high level which is maintained throughout the infusion. However, none of the animals showed any serious complications other than only minimal oozing of blood from the fresh operative wound in the femoral area which was created for venipuncture.

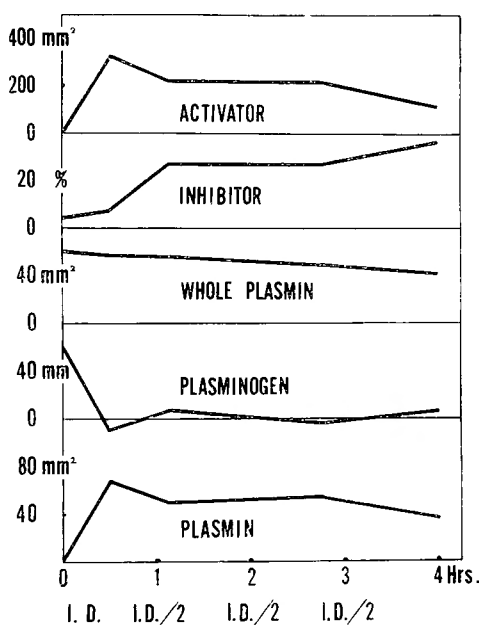


Fig. 10. Changes in fibrinolytic activity in animals treated with small dosage of SK. (Fibrin plate method).

(Group 2) : Repeated treatment with small dosage of SK. for 3 days.

Table 7 shows the results of therapy in this group.

Three out of 6 clots were lysed almost completely when treated within 1 hour, while only 1 out of 6 clots was dissolved when treated on the 2nd day. The percentage of circulation restored of vessels treated within 1 hour was 50.0 per cent, while only 16.7 per cent on the 2nd day.

Complications were rare and minimal in this group, and biochemical changes were

**Table 7** Results of repeated treatments with small dosage of SK. for 3 days (Group 2)

Time of Therapy	No. of Cases	Grade of Occlusion					Percentage of Circulation restored
		IV.	III.	II.	I.	0.	
Within 1 hour	6	1	2		2	1	50.0%
2nd day	6	1	1			1	16.7%

similar to those in group 1.

(Group 3) : Intravenous injection of larger dosage of SK.

Table 8 shows the results of therapy in this group.

Ten clots were treated within 1 hour of formation. Of these, 5 were dissolved completely and 1 was partially lysed to grade II. Another 10 vessels were treated on the 2nd day and 4 dissolved completely. In additional 12 vessels treated on the 3rd day, complete lysis was noted in only 2. The percentages of circulation restored of vessels with clots treated within 1 hour, on the 2nd day and 3rd day were 60.0, 40.0 and 16.7 per cent, respectively.

Table 8 Results of treatment with larger dosage of SK. (Group 3)

Time of Therapy	No. of Cases	Grade of Occlusion					Percentage of Circulation restored
		IV.	III.	II.	I.	0.	
Within 1 hour	10	3	1	1		5	60.0%
2nd day	10	6				4	40.0%
3rd day	12	9	1			2	16.7%

Figure 11 shows changes in fibrinolytic activity and fibrinogen levels in animals treated with this dose of SK. As shown, plasma activator and plasmin concentration increase with the reduction of euglobulin lysis time. At the same time both fibrinogen level and mA in thrombelastogram begin to decrease, following which moderate oozing of blood usually ensues from the fresh operative wound.

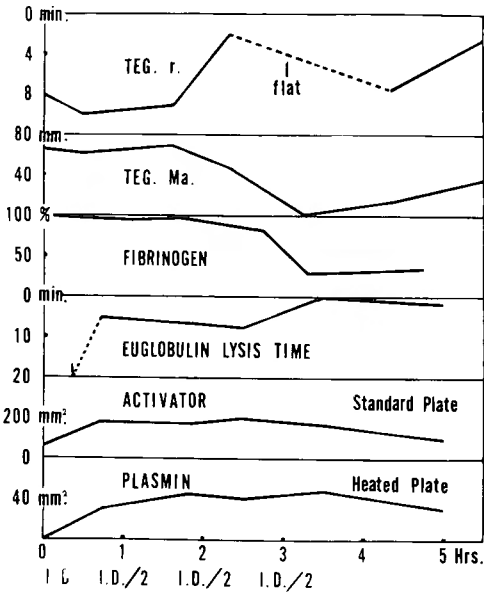


Fig. 11. Changes in fibrinolytic activity and fibrinogen levels in animals treated with larger dosage of SK.

(Group 4) : Combined use of repeated small dosage of SK. and heparin.

Table 9 shows the results of this therapy.

Four clots were treated within 1 hour of formation. Of these, 3 were completely dissolved and 1 was found to be occluded. Of 6 clots treated on the 2nd day, complete lysis was noted in only 1 and the remaining 5 were not affected. The percentage lysis was 75.0 per cent when treated within 1 hour, while 16.7 per cent on the 2nd day.

**Table 9** Results of treatment with repeated small dosage of SK. and heparin (Group 4)

Time of Therapy	No. of Cases	Grade of Occlusion					Percentage of Circulation restored
		IV.	III.	II.	I.	0.	
Within 1 hour	4	1				3	75.0%
2nd day	6	5				1	16.7%

Complications were minimal with this combination therapy.

(Group 5) : Combined use of larger dosage of SK. and heparin.

Table 10. shows the results of this therapy.

Of the 4 clots treated on the 2nd day, 3 were found to be patent and 1 with mural thrombus of grade I. Of 6 clots treated on the 3rd day, complete lysis was noted in 3. The percentage lysis was 100 per cent on the 2nd day, while 50.0 per cent on the 3rd day.

**Table 10** Results of treatment with larger dosage of SK. and heparin (Group 5)

Time of Therapy	No. of Cases	Grade of Occlusion					Percentage of Circulation restored
		IV.	III.	II.	I.	0.	
2nd day	4				1	3	100%
3rd day	6	3				3	50.0%

None of these animals showed any serious reactions other than moderate oozing of blood from the fresh wounds. All animals survived in good health until the time they were sacrificed.

## SERIES II. : SURGICAL TREATMENTS.

(Group 6) : Thrombectomy.

The results of this therapy are shown in Table 11.

Ten vessels were submitted to thrombectomy within 1 hour after clot formation. Of these, 5 showed complete occlusion. Nine vessels were treated on the 2nd day and 5 of

**Table 11** Results of thrombectomy (Group 6)

Time of Operation	No. of Cases	Patent Vessel		Occluded Vessel		Percentage of Circulation restored
Within 1 hour	10	5		5		50.0%
2nd day	9	4		5		44.4%
3rd day	7	1		6		14.3%



them were found to be occluded. Another 7 were treated on the 3rd day. Of these, 5 showed bleeding from arterial ruptures several days after thrombectomy. Only 1 was found to be patent. Thus, the percentages of circulation restored of vessels treated within 1 hour, on the 2nd and 3rd day were 50.0, 44.4 and 14.3 per cent, respectively.

(Group 7) : Coating with EDH -Adhesive after thrombectomy.

Table 12 shows the results of this therapy.

Nineteen vessels were submitted to thrombectomy and then coated with EDH-Adhesive. The vessels were entirely prevented from rupturing by the coating, but a significant decrease was noted in the percentage of circulation restored. The percentages of circulation restored of vessels treated within 1 hour, on the 2nd and 3rd day were 25.0, 16.7 and 0 per cent, respectively.

**Table 12** Results of Coating with EDH-Adhesive after thrombectomy (Group 7)

Time of Operation	No. of Cases	Patent Vessel	Occluded Vessel	Percentage of Circulation restored
Within 1 hour	4	1	3	25.0%
2nd day	6	1	5	16.7%
3rd day	9	0	9	0%

(Group. 8) : Anticoagulation with heparin after thrombectomy.

Table 13 shows the results of this therapy.

Seventeen vessels were included in this study. The percentages of circulation restored of vessels treated within 1 hour, on the 2nd and 3rd day were 50.0, 60.0 and 16.7 per cent, respectively.

**Table 13** Results of anticoagulation with heparin after thrombectomy (Group 8)

Time of Operation	No. of Cases	Patent Vessel	Occluded Vessel	Percentage of Circulation restored
Within 1 hour	6	3	3	50.0%
2nd day	5	3	2	60.0%
3rd day	6	1	5	16.7%

(Group 9) : Intramuscular administration of SK. after thrombectomy.

Of 6 vessels treated on the 2nd day, circulation was restored in only 1 (Table 14).

**Table 14** Results of intramuscular administration of SK. after thrombectomy (Group 9)

Time of Operation	No. of Cases	Patent Vessel	Occluded Vessel	Percentage of Circulation restored
2nd day	6	1	5	16.7%

(Group 10) : Combined use of SK. and heparin after thrombectomy.

The results are shown in Table 15.

Fourteen arteries were used. Of these, 7 were treated on the 2nd day and 6 of them were found to be open. The remaining 7 were treated on the 3rd day but circulation was restored in only 2. The percentage of patent arteries was 85.7 per cent when treated on the 2nd day and 28.6 per cent on the 3rd day.

**Table 15** Results of combined use of SK. and heparin after thrombectomy (Group 10)

Time of Operation	No. of Cases	Patent Vessel	Occluded Vessel	Percentage of Circulation restored
2nd day	7	6	1	85.7%
3rd day	7	2	5	28.6%

## DISCUSSION

### I. FIBRINOLYTIC TREATMENT.

Several problems should be elucidated before the fibrinolytic enzymes are used in clinical cases, especially with carotid and/or cerebral thrombosis. These include 1) dosage and mode of applications, 2) timing of administration, 3) complications and 4) combined use with anticoagulant drugs and/or surgery, etc.

#### 1) Dosage and mode of applications.

SHERRY et al<sup>9)10)</sup>. reported that thrombolytic states of long duration and of great intensity could be produced in man by the intravenous infusion of streptokinase, using an initial streptokinase dose calculated by multiplying 20 minutes test end point by the patient's total blood volume.

Treatment with small dosage of SK. in the present study was scheduled according to their method. However, results of this treatment were unsatisfactory even with freshly formed clots, though fibrinolytic activity in animals maintained a relatively high level throughout the infusion. These poor results were probably due to; 1) difference in fibrinolytic enzyme systems between human and dog and 2) the fact that thrombi used in this study are platelet thrombi produced in arteries, which usually occlude vessels completely within 48 hours and which appear to be less susceptible to fibrinolytic therapy than other thrombi.

Then, another 2 methods of SK. administration were also attempted; 1) repeated administration of small dosage of SK. for 3 days and 2) administration of larger dosage of SK. The results of freshly formed clots were improved to be 50.0 per cent in the former and 60.0 per cent in the latter. Thus, so far as the freshly formed clots are concerned, almost the same results may be obtained by either of these 2 methods.

However, the dissolution rate of the repeated treatment with small dosage of SK. decreased markedly when treated on the 2nd day of clot formation, while a relatively high dissolution rate was consistently obtained by the treatment with larger dosage of SK. even on the 2nd day. These results indicate that thrombolysis of older clots cannot be expected otherwise than by treatment with larger dosage of SK.

#### 2) Timing of administration.

It is well known that the timing of administration is essential for successful fibrinolytic therapy. BACK and AMBRUS<sup>9)</sup> demonstrated in dogs that, even in vein, clots older than

Table 16 Summary of results of fibrinolytic treatment

Gp.	Clot Age	Within 1 hour	2nd day	3rd day
	Mode of Therapy			
I.	Small Dose of SK.	1/6 (16.7%)	—	—
II.	Daily Small Dose	3/6 (50.0%)	1/6 (16.7%)	—
III.	Larger Dose of SK.	6/10 (60.0%)	4/10 (40.0%)	2/12 (16.7%)
IV.	Repeated Small Dose with Heparin	3/4 (75.0%)	1/6 (16.7%)	—
V.	Larger Dose with Heparin	—	4/4 (100%)	3/6 (50.0%)

3 days were not affected by human plasmin injections while those less than 3 days old were completely dissolved.

The present study also confirms that the percentage of dissolution decreases as the age of the clot increases, and clots older than the 3rd day are not affected even by the larger dosage of SK. The growth of the clot from mural to complete occluding clot within 48 hours may probably contribute to the resistance to lysis by reducing the contact surface between the clot and enzymes.

It is apparent from these observations that the treatment should be started as soon as possible after the onset of clot formation.

### 3) Complications.

There is no doubt from the above observations that the fibrinolytic treatment is also effective even on the complete occluding thrombosis produced in the carotid arteries in dogs. However, it is also true that fibrinolytic treatment is frequently accompanied by some changes in the blood coagulation mechanism. Therefore, it might be supposed that hemorrhage intracranially and/or elsewhere in the body would be induced or intensified by this therapy.

Regarding the possibility of the intracranial hemorrhage, J. HANDA<sup>12)</sup> demonstrated in dogs that enhanced fibrinolytic activity did not induce or intensify the hemorrhage into the fresh cerebral softening foci. These observations were also confirmed by a series of experiments in 9 dogs in the present study.

An electric clot was produced at the bifurcation of the common carotid artery after clipping of the ipsilateral posterior communicating and anterior cerebral arteries in 9 dogs. Of these, 5 were served as controls and the remaining 4 animals received the infusion of larger dosage of SK. soon after the procedures. Cerebral softenings occurred in the territory of the ipsilateral middle cerebral artery in 3 out of 5 controls and in 3 of 4 in the treated group. The mean ratio of the width of infarction area to the whole brain of the treated group was proved to be considerably low as compared with the controls as shown in Table 17. Moreover, animals of the treated group did not show any evidence of increased hemorrhage into the softening foci. These results seem to indicate that intracranial hemorrhage may not be induced or intensified even by the treatment with larger dosage of SK.

On the other hand, a mild to moderate oozing of blood from the fresh wound was frequently observed in animals treated with larger dosage of SK., though all animals survived in good health and no evidence of hemorrhage was found in the organs at autopsy.

**Table 17** Effect of treatment with larger dosage of SK. on the prevention of cerebral infarction. Note a marked decrease in the mean percentage of infarction in treated group.

	No. dogs Employed	No. dogs with Symptoms	No. dogs with Infarction	Mean of % Infarction
Control Group	5	3	3	2.47%
Treated Group	4	0	3	0.49%

Such an unpleasant oozing was never noted in animals treated repeatedly with small dosage of SK. for 3 days.

Thus, so far as the treatment for freshly formed clots is concerned, repeated treatment with small dosage of SK. should be used rather than single treatment with larger dosage of SK.

#### 4) Combined use of fibrinolytic and anticoagulant drugs.

The therapeutic goal in thromboembolism lies not only in clot dissolution but also in the maintenance of vascular patency after clot lysis has been achieved. Recurrences of clot formation may frequently occur after successful clot lysis as far as original thrombogenic stimulus persists.

Since the results of the present experiments were examined not immediately but a week after the initiation of fibrinolytic therapy, the possibility of clot reformation might be unavoidable. To prevent such clot reformation, heparin was used with streptokinase in groups 4 and 5.

Combination therapy of the larger dosage of SK. and heparin resulted in a 100 per cent lysis when treated on the 2nd day and a 50 per cent lysis on the 3rd day. In addition, the risk of occurrence of an unpleasant oozing from the fresh wound was not enhanced by combined use of these drugs. In contrast, results were not so good as were expected except in case of the freshly formed clots in the group treated with small dosage of SK. and heparin for 3 days.

From the above observations, it is apparent that fibrinolytic enzymes should be used in combination with anticoagulant drugs for thromboembolism.

## II. SURGICAL TREATMENT.

There is no doubt that surgical reconstructive operation is the best mode of treatment which assures the immediate restoration of thrombotic or embolic occlusion of the vessels. However, such a reconstructive operation is often unsuccessful even in larger arteries when they are occluded completely. Also, surgery is usually difficult in small arteries especially below 4 mm. in diameter, because of technical difficulties as well as frequent clot reformation after surgery.

The vessels used in the present study were considerably small, ranging in diameter from 2.5 to 4 mm. and the majority of them showed the complete occlusion at the time of operation. Therefore, it may be considered in the present study that good results of surgery cannot be expected without the combined use of anticoagulant and/or fibrinolytic agents.

#### 1) Thrombectomy.

It is well known that the success of operation in cases with thromboembolism is entirely dependent upon the duration of occlusion. The results shown in Table 18 also confirmed this fact. The percentage of circulation restored was 50.0 per cent when treated within 1 hour, 44.4 per cent on the 2nd day and 14.3 per cent on the 3rd day. These results closely resemble those of a group treated with larger dosage of SK. However, in 5 out of 7 cases submitted to thrombectomy on the 3rd day, animals died from arterial rupture within 3 days after operation. These results indicate that fibrinolytic treatment may be preferable rather than surgery in cases with small arteries especially below 4 mm. in diameter.

Table 18 Summary of results of surgical treatment

Gp.	Mode of Therapy	Clot Age	Within 1 hour	2nd day	3rd day
VI.	Thrombectomy		5/10 (50.0%)	4/9 (44.4%)	1/7 (14.3%)
VII.	Thrombectomy with Adh.		1/4 (25.0%)	1/6 (16.7%)	0/9 (0%)
VIII.	Thrombectomy with Heparin		3/6 (50.0%)	3/5 (60.0%)	1/6 (16.7%)
IX.	Thrombectomy with SK.			1/6 (16.7%)	
X.	Thrombectomy with Hep. & SK.			6/7 (85.7%)	2/7 (28.6%)

## 2) The effect of heparin and/or streptokinase after thrombectomy.

As mentioned, the major difficulties in the application of thrombectomy to small arteries has been the danger of arterial rupture and frequent clot reformation after surgery.

Regarding the danger of arterial rupture, EDH-Adhesive<sup>11)17)</sup> which has been reported to be the most effective agent for coating and reinforcing intracranial aneurysms, was applied in the present study to prevent arterial rupture after thrombectomy. By this method, arterial rupture was prevented completely; however, the percentage of occurrence of clot reformation increased, resulting in a poor result of circulation restored: 25.0 per cent when treated within 1 hour, 16.7 per cent on the 2nd day and 0 per cent on the 3rd day.

Since the application of anticoagulants and/or fibrinolytic agents after thrombectomy may not be possible without using a method of preventing arterial rupture, such agents were used in the present study after application of EDH-Adhesive. As shown in Table 18, the results were improved to be 50.0% when treated within 1 hour, 60.0% on the 2nd day in group with heparin, and to be 85.7% on the 2nd day, 28.6% on the 3rd day in group with heparin and SK. These results are better than those of thrombectomy and also indicate that thrombectomy with heparin and SK. is much better than that with heparin only.

## SUMMARY

1) The effects of fibrinolytic, surgical and anticoagulant therapies were studied on the complete occluding thrombus which was produced electrically in the common carotid artery in dogs.

## 2) Fibrinolytic treatment:

a) Streptokinase was given intravenously in 3 different doses or modes of application.

Among them, repeated treatment with small dosage of SK. for 3 days and treatment with larger dosage of SK. were found to be equally effective for freshly formed clots. However, the dissolution rate of the former markedly decreased when clots were treated on the 2nd day of formation, while the latter showed a relatively high rate even on the 2nd day.

b) These 2 methods of SK. administration were found not to induce or intensify the hemorrhage into cerebral softening foci. However, a mild to moderate oozing of blood from the fresh wound was frequently observed in a group treated with larger dosage of SK. In contrast, there were no such unpleasant complications in a group treated with small dosage of SK. for 3 days.

c) Anticoagulant drug, especially heparin, was able to increase the percentage of restored circulation by preventing clot reformation after successful clot lysis.

### 3) Surgical treatment :

a) Since vessels used for this study were confined to small arteries and most of them were completely occluded at the time of operation, re-occlusion was frequently observed after thrombectomy.

b) The percentages of restored circulation of the group submitted to thrombectomy closely resembled those treated with larger dosage of SK. However, arterial rupture frequently occurred in the former. Thus, fibrinolytic treatment appears to be the better choice rather than thrombectomy for embolic occlusions in small arteries.

c) Such an arterial rupture was entirely prevented by coating with EDH-Adhesive, but a marked decrease in the percentage of circulation restored was noted.

d) Intramuscular administration of heparin with streptokinase was found to be the most effective method for preventing reocclusion after thrombectomy.

### 4) From these observations it may be concluded that :

a) Freshly formed clots should be treated repeatedly with small dosage of SK.

b) For older clots treatment with larger dosage of SK. is preferable.

c) Heparin should be used in combination with fibrinolytic treatment.

d) The major difficulties in the application of thrombectomy to small arteries are the danger of arterial rupture and frequent clot reformation after surgery. EDH-Adhesive should be used to prevent the former, and heparin with small dose of SK. should be used to prevent the latter.

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## 和 文 抄 録

## 犬に於ける頸動脈血栓の作成並びに治療に関する実験的研究

## —特に線維素溶解療法の応用について—

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頭蓋外或いは頭蓋内動脈血栓症に対して、現今手術療法又は抗凝固剤療法が専ら行われているが、前者は完全閉塞では成績が悪く、後者はincipient stroke 以外には効果がないといわれている。これらの欠点を補う為に実験的に犬の総頸動脈に血栓を作成し、これに対し血栓溶解療法及び手術療法を行ない、その効果を比較検討した。

1) 頸動脈血栓作成法：Chemical Intimectomy, Mechanical Intimectomy, Thrombin Injection 及び通電法の4方法を検討した結果、通電法即ち血管壁に3 mAの微小電流を1時間に亘り通電する方法では90.9%の高率に血栓を作成出来、しかも本血栓は作成3日目以後では完全閉塞を来す血小板血栓である事を見出した。

2) 線維素溶解療法：通電法による血栓作成後、直後・2日目・3日目に種々の方法による線溶療法を行ない、次の結果を得た。

a) 直後に關しては少量の Streptokinase を3日間連続投与する方法及び大量の SK 単独療法は同程度に有効であるが、2日目になると前者の血栓溶解率が低下するのに反し、後者ではなお有効であつた。

b) 3日目を境にして両者共にその成績は著明に低下する。即ち、線溶療法は早期に行なう程成績がよい。

c) 上記2つの SK 投与法は何れも既成の脳軟化巣

内への出血性を増加しない。更に、少量の SK を3日連続投与する方法では創面よりの出血等の副作用が非常に少ないが、大量の SK 投与法では新鮮手術創の oozing を来す事がある。

d) 線溶療法と同時に Heparin の投与を48時間持続する事により、3日目の血栓でも50%の高率に溶解し得た。

3) 手術療法：血栓作成直後・2日目・3日目に、それぞれ血栓剔出術を単独で、或いは EDH-Adhesive, Heparin 又は SK の併用のもとに行ない、次の結果を得た。

a) 血栓剔出術を単独で行なつた場合、術後しばしば再血栓の発生を見た。これは犬の頸動脈が直径2.5～4 mmの細小血管であり、しかも殆どの例が手術時、閉塞性血栓を示している事に基因すると考えられる。

b) 剔出術のみを行なつた群は、大量の SK 投与の群と血管再開率が殆ど同じであつた。更に手術群で、しばしば術後数日して血管の破綻を来した事を考えると、細小動脈では手術療法より線溶療法の方がむしろすぐれていると思われる。

c) この様な動脈破綻は EDH-Adhesive により防ぐ事が出来たが、この際血管再開率は著明に低下した。

d) 血栓剔出術後に Heparin と SK を併用投与する方法は、再血栓の防止に有効であり、術後 Heparin のみを用いる方法より高率の血管再開率を示した。